

What is claimed is:

1. A polynucleotide sequence encoding a fusion protein and comprising, in the 5' to 3' direction and in the same open reading frame:
 - a) a sequence encoding the clover yellow vein virus Nuclear Inclusion a protein, or a mutant or variant thereof having similar proteolytic specificity to that of clover yellow vein virus Nuclear Inclusion a protein;
 - b) a sequence encoding a cleavage peptide recognizable by and cleavable by said clover yellow vein virus Nuclear Inclusion a protein, or said mutant or variant thereof; and
 - c) at least one sequence encoding a polypeptide.
2. The polynucleotide sequence of claim 1, which is DNA.
3. The polynucleotide sequence of claim 2, which is in the form of a double strand.
4. An antisense polynucleotide sequence corresponding to the polynucleotide sequence of claim 1.
5. The polynucleotide sequence of claim 1, in which the sequence of c) encodes more than one polypeptide and there are encoded further cleavage sequences between each encoded polypeptide.
6. The polynucleotide sequence of claim 5, in which said further cleavage sequences are recognizable by clover yellow vein virus Nuclear Inclusion a.
7. The polynucleotide sequence of claim 1, wherein the sequence of c) encodes only one polypeptide.

8. The polynucleotide sequence of claim 1, in which the sequence of b) is wholly or partly comprised in either or both of the sequences a) and c).

9. The polynucleotide sequence of claim 8, in which the sequence of b) is wholly comprised in the sequences of a) and b).

10. The polynucleotide sequence of claim 1, wherein there are encoded one or more amino acid residues between the cleavage peptide encoded by the sequence of b) and the polypeptide encoded by the sequence of c).

11. The polynucleotide sequence of claim 1, in which cleavage of the fusion protein encoded thereby by the Nuclear Inclusion a protein encoded by the sequence of a) yields a polypeptide having an additional N-terminal amino acid sequence of which the terminal residue is a residue of glycine, serine or alanine.

12. The polynucleotide sequence of claim 1, in which cleavage of a fusion protein encoded thereby by the Nuclear Inclusion a protein encoded by the sequence of a) yields a polypeptide having an additional N-terminal amino acid sequence of which the terminal residue is a residue of glycine, serine or alanine, said residue being removable by the action of a suitable aminopeptidase if desired.

13. The polynucleotide sequence of claim 1, in which a proline residue is encoded between the N-terminal of the polypeptide and the C-terminal of the cleavage peptide.

14. The polynucleotide sequence of claim 1, in which a proline residue is encoded between the N-terminal of the polypeptide and the C-terminal of the cleavage peptide,

thereby permitting the cleavage of any residues extending beyond the proline residue by the action of aminopeptidase P (3.4.11.9).

15. The polynucleotide sequence of claim 14, in which, after cleavage up to the the proline residue, the proline residue can then be removed by the action of proline iminopeptidase (3.4.11.5).

16. The polynucleotide sequence of claim 1, in which an alanine residue is encoded between the N-terminal of the polypeptide and the C-terminal of the cleavage peptide.

17. The polynucleotide sequence of claim 1, in which an alanine residue is encoded between the N-terminal of the polypeptide and the C-terminal of the cleavage peptide, thereby permitting the cleavage of any residues extending beyond the alanine residue by the action of aminopeptidase P (3.4.11.9), and cleavage of the alanine residue by the catalytic action of alanine aminopeptidase (3.4.11.14).

18. The polynucleotide sequence of claim 1, wherein the sequence of a is given by nucleotide numbers 10 to 1311 in sequence ID number 1 in the sequence listing.

19. The polynucleotide sequence of claim 1, wherein the sequence of a) encodes a polypeptide given by amino acid numbers 4 to 437 in sequence ID number 2 in the sequence listing.

20. A polynucleotide sequence encoding the clover yellow vein virus Nuclear Inclusion a protein as given by nucleotide numbers 10 to 1311 in sequence ID number 1 in the sequence listing, or a mutant or variant thereof having similar proteolytic specificity to that of clover

yellow vein virus Nuclear Inclusion a protein.

21. A vector containing the polynucleotide sequence of claim 1.

22. The vector of claim 21, which is an expression vector.

23. A host transformed with the vector of claim 21.

24. A host transformed with the vector of claim 22.

25. An expression system comprising a host transformed with the vector of claim 22.

26. A polypeptide expressed by the system of claim 25.

27. A system for the preparation of a polypeptide, wherein a precursor form of the polypeptide is cleaved by clover yellow virus Nuclear Inclusion a protein, or a mutant or variant thereof.

28. The system of claim 27, wherein said precursor is a fusion protein.

29. A system for the preparation of a polypeptide, wherein a precursor form of the polypeptide containing the sequence AsnCysSerPheGlnX is cleaved by clover yellow virus Nuclear Inclusion a protein, or a mutant or variant thereof.

30. A system for the preparation of a polypeptide, wherein a precursor form of the polypeptide containing the sequence AsnCysSerPheGlnX is cleaved by clover yellow virus Nuclear Inclusion a protein, or a mutant or variant thereof, to yield the mature form of the

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polypeptide.

31. The polynucleotide sequence of claim 1, which shares at least 90% sequence homology with residues 10 to 1311 in sequence ID number 1.

32. The polynucleotide sequence of claim 1, further encoding a leader sequence upstream of the sequence a).

33. A fusion protein encoded by a polynucleotide sequence comprising, in the 5' to 3' direction and in the same open reading frame:

- a) a sequence encoding the clover yellow vein virus Nuclear Inclusion a protein, or a mutant or variant thereof having similar proteolytic specificity to that of clover yellow vein virus Nuclear Inclusion a protein;
- b) a sequence encoding a cleavage peptide recognizable by and cleavable by said clover yellow vein virus Nuclear Inclusion a protein, or said mutant or variant thereof; and
- c) at least one sequence encoding a polypeptide.

34. The fusion protein of claim 33 comprising more than one polypeptide and wherein there are located cleavage peptides between each polypeptide.

35. The fusion protein of claim 34, in which said further cleavage sequences are recognizable by clover yellow vein virus Nuclear Inclusion a.

36. The fusion protein of claim 33, which consists of only one polypeptide in addition to the clover yellow vein virus Nuclear Inclusion a protein, or the mutant or variant thereof.

37. The fusion protein of claim 33, wherein the cleavage

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peptide is wholly or partly comprised in either or both of the peptides as encoded by sequences a) and c).

38. The fusion protein of claim 33, wherein the cleavage peptide is wholly comprised in the peptides as encoded by sequences a) and c).

39. The polynucleotide sequence of claim 33, wherein one or more amino acid residues are located between the cleavage peptide and the polypeptide.

40. The fusion protein of claim 33, wherein autolysis by the peptide encoded by sequence a) yields a polypeptide having an additional N-terminal amino acid sequence of which the terminal residue is a residue of glycine, serine or alanine.

41. The expression system of claim 25, wherein autolysis by the peptide encoded by sequence a) yields a polypeptide having an additional N-terminal amino acid sequence of which the terminal residue is a residue of glycine, serine or alanine, and an aminopeptidase in the host serves to remove said N-terminal residue.

42. The fusion protein of claim 33, in which a proline residue is located between the N-terminal of the polypeptide and the C-terminal of the cleavage peptide.

43. The expression system of claim 25, wherein autolysis by the peptide encoded by sequence a) yields a polypeptide having an additional N-terminal proline residue and 0, 1 or a plurality of further amino acid residues, and wherein any said additional amino acid residue beyond said proline is removed by aminopeptidase P (3.4.11.9).

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44. The expression system of claim 43, wherein, after cleavage up to the the proline residue, the proline residue is then removed by the action of proline iminopeptidase.

45. The fusion protein of claim 33, in which an alanine residue is located between the N-terminal of the polypeptide and the C-terminal of the cleavage peptide.

46. The expression system of claim 25, wherein autolysis by the peptide encoded by sequence a) yields a polypeptide having an additional N-terminal alanine residue and 0, 1 or a plurality of further amino acid residues, and wherein any said additional amino acid residue beyond said alanine is removed by aminopeptidase P (3.4.11.9), and the alanine residue is removed by the action of alanine aminopeptidase (3.4.11.14).

47. A polypeptide having the sequence given by residue numbers 4 to 437 in sequence ID number 2 in the sequence listing, or a mutant or variant thereof.

SuS A
48. A polypeptide having the sequence given by residue numbers 4 to 437 in sequence ID number 2 in the sequence listing.

49. A polynucleotide sequence encoding a polypeptide which comprises the amino acid sequence of amino acid numbers 1 to 526 of sequence ID number 12, or which encodes a mutant or variant of said polypeptide, provided that the polypeptide encoded by the polynucleotide sequence is capable of reducing dichloroindophenol and oxidized glutathione.

50. The polynucleotide sequence of claim 49, which shares 55% sequence homology, or more, with amino acid

0 9 8 4 2 3 4 7 6 7 6 5 4

numbers 1 to 526 of sequence ID number 12.

51. The polynucleotide sequence of claim 49, which shares in excess of 70% sequence homology with amino acid numbers 1 to 526 of sequence ID number 12.

52. The polynucleotide sequence of claim 49, which shares in excess of 80% sequence homology with amino acid numbers 1 to 526 of sequence ID number 12.

53. The polynucleotide sequence of claim 49, wherein the coding sequence comprises the nucleotide sequence 70 to 1647 indicated in sequence ID number 11.

54. The polynucleotide sequence of claim 49, which encodes a polypeptide having the sequence -23 to 526 of sequence ID 12, or a mutant or variant thereof.

Suk *PA* 55. The polypeptide encoded by the polynucleotide sequence of claim 49.

56. The polypeptide encoded by the polynucleotide sequence of claim 50.

57. The polypeptide encoded by the polynucleotide sequence of claim 51.

58. The polypeptide encoded by the polynucleotide sequence of claim 52.

59. The polypeptide encoded by the polynucleotide sequence of claim 53.

Suk *PA* 60. The polypeptide encoded by the polynucleotide sequence of claim 54.

SAC A

61. A method for the prophylaxis or treatment of conditions caused by, or related to, oxidative stress, or any disease caused by activated oxygen, comprising the administration to a mammal in need thereof an effective, non-toxic dose of a peptide encoded by the polynucleotide sequence of claim 49.

62. The polypeptide encoded by the polynucleotide sequence of claim 49 for use in the prophylaxis or therapy of arteriosclerosis, diabetes, or ischemic disorders.

SAC X

63. A method for the prophylaxis or treatment of arteriosclerosis, diabetes, ischemic disorders, edema, vascular hyperpermeability, inflammation, gastric mucosa disorders, acute pancreatitis, Crohn's disease, ulcerative colitis, liver disorders, Paraquat's disease, pulmonary emphysema, chemocarcinogenesis, carcinogenic metastasis, adult respiratory distress syndrome, disseminated intravascular coagulation, cataracts, premature retinopathy, auto-immune diseases, porphyremia, hemolytic diseases, Mediterranean anemia, Parkinson's disease, Alzheimer's disease, epilepsy, ultraviolet radiation disorders, radioactive disorders, frostbite or burns, comprising the administration to a mammal in need thereof an effective, non-toxic dose of a peptide encoded by the polynucleotide sequence of claim 49.

64. A pharmaceutical composition comprising a pharmaceutically active amount of the peptide encoded by the polynucleotide sequence of claim 49 together with a pharmaceutically acceptable carrier therefor.

65. A vector containing the polynucleotide sequence of claim 49.

66. An expression vector containing the polynucleotide

sequence of claim 49.

67. A host transformed with the vector of claim 65.

68. A host transformed with the vector of claim 66.

69. An expression system comprising the host of claim 68.

70. The polypeptide produced by the expression system of claim 69.

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71. An antibody or an equivalent thereof, which specifically recognizes KM31-7 protein, or which specifically recognizes a mutant or variant of KM31-7 protein.

72. The antibody of claim 71 which is a monoclonal antibody.

July 10
73. The antibody of claim 71, wherein said antibody antigenically resembles a human antibody.

74. An anti-idiotype antibody recognizing the recognition site of the antibody of claim 71.

75. The antibody of claim 71, as produced by the hybridoma designated MKM150-2 and deposited at the Fermentation Research Institute of the Agency of Industrial Science and Technology, Japan, under the deposit number FERM BP-5086, or an antibody derived from a hybridoma obtained from the hybridoma designated MKM150-2.

76. The antibody of claim 71, for use in the isolation and purification of KM31-7 protein.

Sub A¹¹

77. A process for the purification of KM31-7 protein comprising the use of the antibody of claim 71 to bind said protein.

78. A hybridoma which expresses the antibody of claim 71 in culture.

79. The hybridoma designated MKM150-2 and deposited at the Fermentation Research Institute of the Agency of Industrial Science and Technology, Japan, under the deposit number FERM BP-5086.

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80. A polypeptide comprising the sequence given by residues 4 to 437 in sequence ID number 2, or a mutant or variant thereof.

81. DNA which hybridizes with DNA having the nucleotide sequence as given by numbers 10 to 1311 in sequence ID number 1 in the sequence listing, and wherein the corresponding sense strand encodes a protein having protease activity.

82. The DNA according to Claim 81, wherein the conditions of hybridization use 6x SSC at 60 to 70°C.

83. The expression system of claim 25, wherein the host is Escherichia coli.

84. The expression system of claim 69, wherein the host is Escherichia coli.

85. DNA which hybridizes with the polynucleotide sequence of claim 49, and wherein the corresponding sense strand encodes a polypeptide having reducing activity.

86. The DNA of Claim 85, wherein the conditions of hybridization use 6x SSC at 60 to 70°C.

87. The polynucleotide sequence of claim 1, wherein the polypeptide encoded by the sequence of c) is a polypeptide which comprises the amino acid sequence of amino acid numbers 1 to 526 of sequence ID number 12, or is a mutant or variant of said polypeptide, provided that the polypeptide encoded by the polynucleotide sequence is capable of reducing dichloroindophenol and oxidized glutathione.

88. The fusion protein encoded by the polynucleotide sequence of claim 87.

Ado
X¹³